Ligation protocol (follows iGEM official protocol)

1. Calculate the amount of insert as $\frac{\text{vector(ng)}}{\text{vector length}} : \frac{\text{insert(ng)}}{\text{insert length}}$ = 1:1, let the vector amount = 25 ng.
2. Add the materials as follow:

T4 DNA ligase	0.5 ul
Ligase buffer	1 ul
Vector (25 ng)	x ul
Insert A	follow the result you calculates
Insert B	follow the result you calculates
ddH2O	to final volume 10 ul
	10 ul

3. Mix and centrifuge the reaction mixture.
4. Put the reaction under { room temperature for 2~3 hours / 16 ℃ over night (30 mins?? as official protocol)
5. Heat-kill in 80℃ for 20 mins. (This step can be ignored.)
6. Transform 1~2 ul of the product.

Ligation protocol:

1. Calculate the amount of insert as $\frac{\text{vector(ng)}}{\text{vector length}} : \frac{\text{insert(ng)}}{\text{insert length}}$ = 1:1 (or 1:3 or 1:5 or 1:7), let the vector amount = 50 ng.
2. Add the materials as follow:

T4 DNA ligase	1 ul
Ligase buffer	2 ul
Vector (25 ng)	x ul
Insert A	follow the result you calculates
Insert B	follow the result you calculates
ddH2O	to final volume 10 ul

10 l

3. Mix and centrifuge the reaction mixture.
4. Put the reaction under { room temperature for 2~3 hours / 16 ℃ over night }
5. Transform 5~10 ul of the product.